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Cryopreservation of Semen From Adolescent Patients With Malignancies

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In adult oncological patients semen cryopreservation offers the possibility of preserving fertility prior to aggressive therapy that may lead to infertility. The cryopreserved semen can later be used to induce pregnancies in the partner by techniques of assisted fertilization. In adolescent boys the question of fertility is often beyond consideration when the young patient's life is threatened acutely. However, improved survival rates increasingly prompt the question of quality of life after therapy, including fertility. Semen quality is known to be impaired in patients with malignancies and may be further impaired by the process of cryopreservation. Since normal values for semen in adolescents are not known and spermatogenesis may be impaired by the malignant disease, it was unclear whether semen samples from adolescents with malignancies warrant cryopreservation at all. In order to demonstrate the feasibility of semen cryopreservation in adolescent males, we compared the results from 12 pubertal boys aged 14–17 years with those from 17 young adults aged 18–20 years who had similar malignancies and, additionally, to 210 adults with malignancies (>20 years). Luteinizing hormone serum values were significantly lower in adolescents than in adult patients. Follicle stimulating hormone showed a significant increase with age. Testosterone serum levels and testicular volumes showed similar distribution patterns in adolescent and adult men. Sperm concentrations, sperm motility, and normal sperm morphology in the adolescent patients did not show significant differences compared with adults. Thus cryopreservation of semen should be considered as an option to young male patients whose cancer therapy will include potentially gonadotoxic treatment.

Key words: semen cryopreservation, fertility, childhood cancer, testicular volume, FSH

INTRODUCTION

Due to effective chemotherapeutic and radiotherapeutic intervention, about 70% of children with oncological diseases survive their malignancies. While late effects of aggressive therapy on different organ systems, for example, the skeleton, thyroid gland, heart, lung, and nervous system, are under investigation, it is difficult to foresee the implications for reproductive organs [1,2]. However, most cancer therapies affect gonadal function independent of the patient's pubertal status at the time of treatment [3]. Therefore, survival is often associated with infertility and/or endocrine problems during adulthood [2].

In adult oncological male patients, cryopreservation of semen offers the possibility of preserving spermatozoa prior to surgery, chemotherapy, or radiotherapy [4]. However, for many doctors and parents problems of fertility go beyond their immediate considerations when the life of the young patient is threatened and acute treatment is necessary. Moreover, questions of sexuality and fertility continue to be taboo subjects, and physicians and patients often hesitate to talk about these subjects. Nevertheless, the quality of life of a cured adolescent cancer patient may also depend on fertility, and therefore possible impairment of reproductive functions deserves consideration prior to therapy.

Malignancies are often associated with sperm variables below the normal range prior to any therapy potentially toxic to the gonads [5,6]. So far, no normal values of semen in adolescents are available. Spermatogenesis may be impaired by the malignant disease itself, as it can

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TABLE I. Patient Groups and Diagnoses

Diagnoses	Boys 14–17 years (n = 12)	Adults 18–20 years (n = 17)	Adults >20 years (n = 210)
Testicular cancer	1	1	104
Hodgkin's disease	3	6	45
Leukemia	2	3	26
Other tumors[a]	6	6	29
Other diseases[b]	0	1	6

[a]Ewing's sarcoma, melanoma, osteosarcoma, rhabdomyosarcoma of the epididymis, colon carcinoma, nephroblastoma, brain tumors, and aplastic anemia.
[b]Lupus erythematosus, histiocytoma, inguinal herniotomy, testicular trauma, and multiple sclerosis.

be in adult men. Up to now it is unclear whether semen samples from adolescent patients warrant cryopreservation at all.

In order to demonstrate the feasibility of semen cryopreservation in adolescent patients with malignancies in comparison with adults, we have evaluated the data on hormones, testicular volumes, and semen parameters from 239 patients with malignancies attending the outpatient clinic of the Institute of Reproductive Medicine of the University for semen cryobanking. The patients included 12 adolescents, aged 14–17 years, and 17 young adults, aged 18–20 years, with malignancies.

PATIENTS AND METHODS

Patient Characteristics

Two hundred and thirty-nine patients aged 14–63 years attended the Institute of Reproductive Medicine of the University of Münster for cryopreservation of sperm. Patient selection depended on the counselling oncologists and the willingness of a patient to consider preservation of fertility by semen cryopreservation as adequate. Especially in the younger boys both the patient and the parents had to decide on the procedure for semen cryopreservation. This selection occurred before patients attended the Institute of Reproductive Medicine for further counselling and treatment. The patients were classified in three patient groups according to age and diagnoses (Table I). Group I consisted of 12 patients aged 14–17 years with a mean age of 15.9 ± 1.0 years (mean ± SD). Seventeen boys aged 18–20 (19.5 ± 1.0 years) formed group II, while group III consisted of 210 adults older than 20 (28.9 ± 5.8) years. Data on the semen characteristics of 135 of the adult patients documented have been included in another publication in order to review the possibilities and limits of cryopreservation of semen in cancer patients [7]. Clinical investigation included the patient's medical history and a clinical examination.

Hormone Determinations

Blood samples were drawn for determination of luteinizing hormone (LH), follicle stimulating hormone (FSH), and testosterone (T) between 0730 h and 1200 h in the morning. LH and FSH were determined by highly specific time-resolved fluoroimmunoassays (Delfia hLH Spec and Delfia hFSH Spec, Pharmacia, Freiburg, Germany). The normal range in our laboratory for LH is 2–10 IU/l, and for FSH it is 1–7 IU/l, with detection limits of 0.25 IU/l and 0.12 IU/l, respectively. Testosterone was determined by radioimmunoassay (RIA), as described earlier [8]. The lower normal limit for T is 12 nmol/l.

Determination of Testicular Volumes

Determination of testicular volumes was done by palpation and comparison with a Prader orchidometer and/or by ultrasonography. Ultrasound volumes were determined by the area-length calculation using a 7.5 MHz sector scanner (Siemens Sonoline SL2, Erlangen, Germany), as published earlier [9]. Total testicular volume is given as the sum of the right and left volumes. For comparative analyses of testicular size, patients with testis tumors were excluded.

Semen Analyses

Semen analyses were preformed according to WHO guidelines [10, 11] after liquefication at 37°C and included the evaluation of sperm concentration, total sperm number, and the motility and morphology of sperm prior to cryopreservation. The lower normal limit for sperm concentration is 20 mill/ml; the lower limit for motility is 50% of progressively motile spermatozoa or 25% of rapidly progressive spermatozoa. During the period of investigation, the normal lower limits for sperm morphology were reconsidered and shifted from 50% initially [10] to 30% morphologically normal spermatozoa [11]. For this investigation the lower normal limit of 30% morphologically normal spermatozoa was set as the standard reference point. Every patient usually provided two to three semen samples to be analyzed and cryopreserved. Altogether 566 semen samples were analyzed. In this study, only the first ejaculate of each patient was considered for evaluation of semen variables.

Cryopreservation of Semen

After semen analysis, samples were processed immediately for cryopreservation under sterile conditions. Semen was mixed rapidly with an equal volume of sterile cryoprotectant (1:1) (Steritec™, Steripharm, Berlin, Germany) and dispensed into straws (maximum volume 500 μl). The straws were put into cassettes and frozen under a protocol using cryopreservation equipment (Planer Kryo 10-Serie II, Messer Griesheim, Krefeld, Germany) [12].

TABLE II. Results of Semen Analysis, Hormone Measurements, and Testicular Volumes*

Parameters (units)	Normal limits	Boys 14–17 years (n = 12)	Adults 18–20 years (n = 17)	Adults >20 years (n = 210)
Semen variables				
Sperm concentration (mill/ml)	≥20	44 ± 21; 13 (11)	40 ± 8; 31 (17)	40 ± 4; 20 (210)
Total sperm number (mill/ejaculate)	≥40	157 ± 94; 46 (11)	127 ± 33; 72 (17)	158 ± 18; 71 (210)
Sperm motility [grade a+b] (%)	≥50	30 ± 7; 23 (11)	45 ± 5; 53 (17)	39 ± 1; 41 (204)
Sperm motility, after freezing and thawing [grade a+b] (%)		18 ± 6; 12 (11)	22 ± 4; 24 (15)	14 ± 1; 12 (188)
Normal morphology (%)	≥30	23 ± 4; 21 (11)	30 ± 4; 32 (16)	24 ± 1; 20 (201)
Hormones				
FSH (IU/l)	1–7	5.2 ± 1.1; 4.5 (10)	4.0 ± 1.0; 3.0 (16)	6.4 ± 0.4; 4.8 (196)
LH (IU/l)	2–10	2.5 ± 0.3; 2.2 (9)	6.9 ± 1.8; 4.9 (16)	5.5 ± 0.3; 4.6 (195)
Testosterone (nmol/l)	>12	12.4 ± 1.3; 12.1 (9)	15.8 ± 1.5; 16.7 (16)	16.2 ± 0.5; 15.1 (194)
Testes				
Testes volumes, right + left (ml)		29.6 ± 2.2; 28.5 (8)	32.6 ± 2.4; 30.5 (14)	36.7 ± 1.0; 37.0 (101)

*Data are given as mean ± SEM, median. Number of patients is in parentheses.

The freezing procedure itself is a critical step in preserving sperm integrity, and thus quality. To decrease sperm damage, it is necessary to use an optimized freezing time and constant storage conditions. Cryopreserved semen samples were stored in liquid nitrogen. After freezing, an aliquot of the semen was thawed and sperm motility was determined. For a period of 3 months cryodepots were stored at the Institute of Reproductive Medicine. Within this time period patients had to decide whether long-term storage of semen samples was desired. In this event, the frozen semen samples were transferred to a commercial cryo bank, with constant storage conditions guaranteed (Messer Griesheim, Krefeld, Germany). Otherwise semen samples were destroyed.

Statistics

Descriptive statistics of untransformed variables are given as the arithmetic mean and the standard error of the mean and, additionally, as the median where indicated. *P* values of <0.05 were considered significant. The homogeneity of the relative frequencies of subjects in different diagnostic groups was tested by the chi-square test. All variables were checked for normal distribution by applying the Kolmogorov-Smirnov one-sample test for goodness-of-fit. The variables LH, FSH, sperm concentration, and total sperm number were log-transformed before analysis to produce the normal distribution. In addition, we check for the variances between groups by using Bartlett's test, and the variances were found to be equal. One-way analysis of variance was applied for testing differences between the three study groups. When the overall *F* test was significant, differences between groups were tested by the a posteriori Duncan multiple comparison test for an unequal sample size at a significance level of $\alpha = 0.05$. Simple regression analysis was applied where appropriate. Analyses were performed using the statistical software Statgraphics, version 5.0 (STSC Inc., Rockville, MD).

RESULTS

The distribution pattern of the diagnoses was comparable in groups I and II and differed from group III due to a large number of patients with testicular tumors (n = 104) and a higher proportion of patients with Hodgkin's disease (n = 45) in group III. Three patients in group I had Hodgkin's disease, two patients had osteosarcoma, and two patients had leukemia. One patient each had a neuroblastoma, a melanoma, an astrocytoma, an embryonic cell carcinoma of the testis, and a rhabdomyosarcoma of the epididymis. In group II, 6 of the 17 patients had Hodgkin's disease, 4 had osteosarcoma, 3 had acute leucemia, one had aplastic anemia, one had Ewing's sarcoma, one had a teratoma of the testis, and one had a histiocytoma (Table I).

Sixty-nine adult patients were married at the time of investigation. Only in group III had 27 adult patients already fathered children before they became ill. Twenty of 27 patients had fathered one child each, 6 patients had 2 children each, and one patient had 3 children prior to

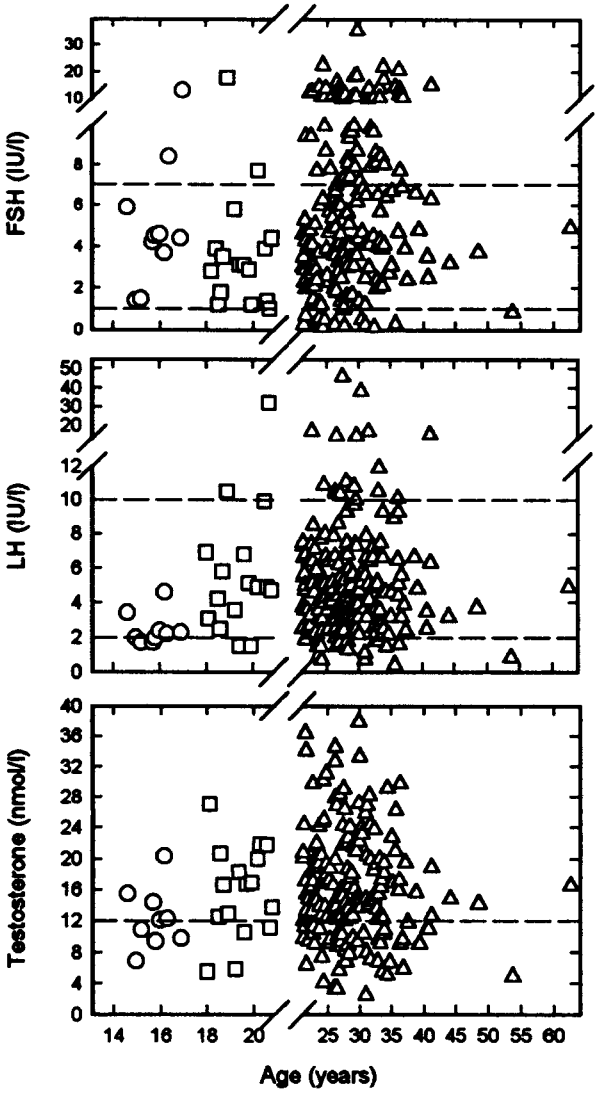

Fig. 1. Individual hormone values in adolescent patients (14–17 years, open circles) and adult patients (18–20 years, open squares; older than 20 years, open triangles) with malignancies prior to cancer treatment. FSH (**upper panel**), LH (**middle panel**), and T (**lower panel**). Broken lines indicate lower (LH, FSH, T) and upper (FSH, LH) normal limits.

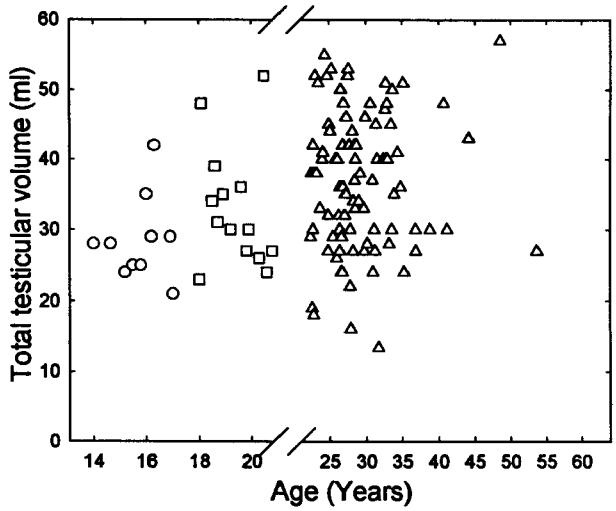

Fig. 2. Individual total (sum of right and left) testicular volumes in adolescent patients (14–17 years, open circles) and adult patients (18–20 years, open squares; older than 20 years, open triangles) with malignancies prior to cancer treatment. Patients with testicular cancer are not included.

illness. Eight patients in group I, 7 patients in group II, and 119 patients in group III stored their semen samples on a long-term basis for possible later use. Thus far 13 inseminations in 5 patients' partners have been performed, resulting in two pregnancies with two live births (twins) and one abortion.

Evaluation of endocrine profiles of all patient groups revealed significantly lower LH serum levels between groups I and II, and between groups I and III ($P < 0.05$), with no difference between groups II and III (Table II, Fig . 1). Testosterone serum levels did not reveal significant differences between groups ($P > 0.05$) (Table II, Fig. 1). FSH serum levels showed an increase with age ($r = 0.19$; $P < 0.05$) (Table II, Fig. 1). FSH serum levels showed an increase with age ($r = 0.19$; $P < 0.05$) (Table II, Fig. 1).

Testicular volumes and semen characteristics, including sperm concentration, total sperm number, forward sperm motility before and motility after freezing and thawing, as well as normal sperm morphology, did not show differences between groups and did not correlate with patient age ($P > 0.05$) (Table II, Figs. 2 and 3). Patients aged 14–17 years did not show significant differences in comparison with adult patients with malignancies. However, the median sperm concentration was below the lower normal limit (Table II). Six patients in group I and six patients in group II produced sperm concentrations below the lower normal limit, although testicular volumes indicated normal testicular development (Table II, Figs. 2 and 3).

It should be mentioned that one adolescent patient and one adult patient of group III did not produce an ejaculate. A 15-year-old boy suffered from osteosarcoma and had low serum testosterone (6.8 nmol/l) with normal gonadotropin levels (LH 2.0 IU/l, FSH 1.4 IU/l). The second patient was 21 years old and had a testicular tumor. He had normal hormone levels (LH 5.5 IU/l, FSH 4.6 IU/l, and testosterone 24.4 nmol/l). He presented shortly after surgery and still suffered from pain. Five patients of group III had azoospermia. Three patients presented with Hodgkin's disease, and one patient each had a metastatic teratoma and an embryonic cell carcinoma of the testis. Only one of the patients with Hodgkin's disease had a subnormal serum testosterone level (10 nmol/l). Azoospermia in the testicular cancer

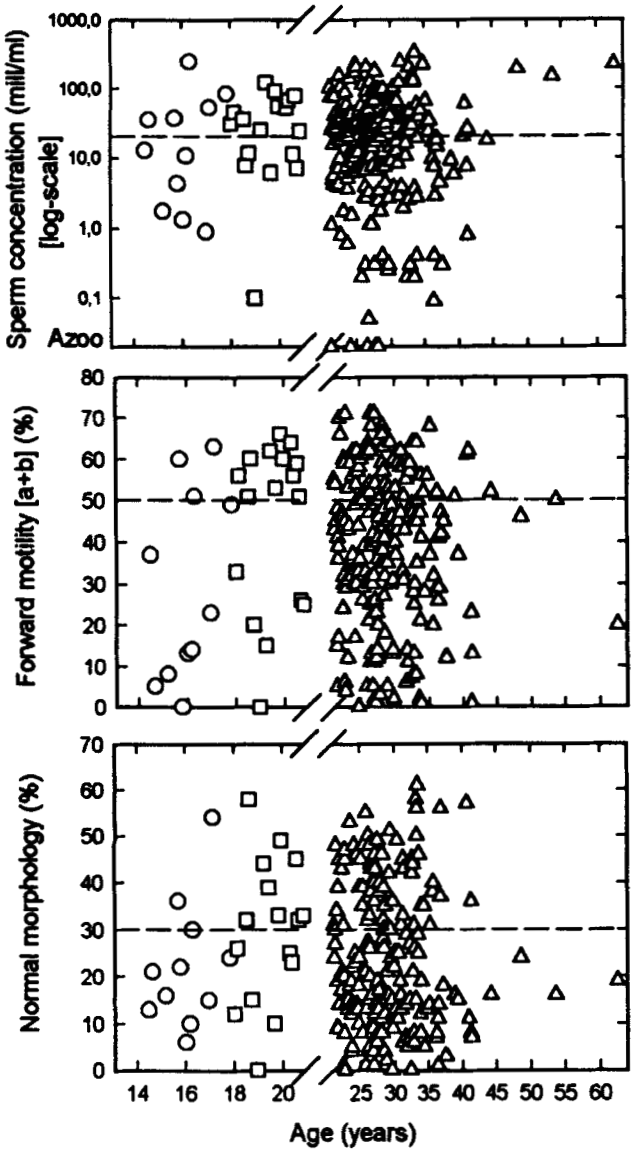

Fig. 3. Individual sperm concentrations (**upper panel**), forward sperm motility (**middle panel**), and normal morphology (**lower panel**) in adolescent patients (14–17 years, open circles) and adult patients (18–20 years, open squares; older than 20 years, open triangles) with malignancies prior to cancer treatment. Note logarithmic scale used for sperm concentrations. Broken lines indicate lower normal limits.

patients may be due to suppressed FSH (0.25 IU/l) and low testosterone (7.4 nmol/l) levels, respectively.

DISCUSSION

In adult male patients with malignant diseases facing a potentially gonadotoxic treatment and loss of fertility, cryopreservation of semen is an accepted preventive therapeutic strategy. By this preventive treatment patients are offered the chance to have or complete their family after finishing the acute treatment of their disease [4,7].

The need for cryopreservation is based on the observation that despite improvement of treatment and the use of less toxic therapeutic agents, testicular damage cannot be completely prevented [13]. A review of the literature since 1980, which includes date on semen variables after childhood cancer (for a review see Table III), showed that in 31 patients treated with non-alkylating agents (such as Adriamycin, vincristine, methotrexate, and 6-mercaptopurine), 84% of patients had a recovery of spermatogenesis to oligozoospermia or normozoospermia, while 16% remained azoospermic 1–11 years after the end of treatment. If cisplatin was used for therapy alone or in combination, 37% of 57 patients remained azoospermic. If alkylating agents, for example, cyclophosphamide, or procarbazine were used in different combinations and in different cumulative doses, 68% of a total of 93 reported cases remained azoospermic 1–20 years after the cessation of therapy (see Table III).

In adolescent boys no data on semen variables characterizing the development of gonadal function, and thus spermatogenesis, are available. By evaluating the relationship between gonadotropin excretion, the increase of testicular volume, secondary sex characteristics, and the determination of sperm in urine, the median age of spermarche is estimated to be 13–14 years of age, with a range between 11–17 years [14–17]. Testicular volumes range 5–20 ml at the time of spermarche [16–18]. A significant increase in testosterone serum levels was observed after the onset of testis growth at the age of 10–12 years. According to published data, the onset of puberty in boys is marked by an increase in gonadotropins and testicular growth. Puberty is regarded to end at the age of 17, when growth finally comes to an end and the epiphyses are closed. The period of pubertal development, including the development of reproductive functions and psychosocial development, is defined as adolescence, although no exact time points are given in the literature [18–20]. Thus we chose patient group I according to the above-mentioned characteristics (aged 14–17 years). Patient group II was selected on the basis of adult age (18–20 years) and diagnoses that are similarly distributed in this age class compared with the adolescent group (14–17 years). In adults older than 20 years, a higher proportion of testicular tumors and Hodgkin's diseases are found compared with the group of adolescents and young adults.

We demonstrated that adolescent patients aged 14–17 years with malignancies show hormone values, testicular volumes, and sperm concentrations within the adult ranges of patients with malignancies. Semen parameters, such as sperm concentration, sperm motility, and normal morphology, did not show significant differences compared with adult patients with malignancies, irrespective of the diagnosis. However, the median sperm concentration was below the lower normal adult range. These are

TABLE III. Reports on Semen Parameters and Pregnancy Outcome in Male Patients with Malignancies During Childhood or Adolescence Since 1980*

Reference	Diagnosis	Therapy	Patients	Results of semen analysis	Pregnancies in the female partner	Time after end of therapy
Aubier et al., 1989 [32]	Solid tumors	Cyclophosphamid or MOPP (>2 cycles); Adriamycin/ actinomycin/vinblastin	23	17 azoospermia 6 normozoospermia	Not reported	Median 9 yr, range 1–20 yr
Blatt et al., 1980 [33]	ALL, AML	Adriamycin, prednisone, daunomycin, methotrexate, vincristine	3		6 (incl. 1 elective and 1 spontaneous abortion)	On chemotherapy, 8 and 16 months, $2\frac{1}{2}$, $3\frac{1}{2}$ and 4 yr;
	ALL, Ewing's sarcoma, reticulum cell sarcoma	Cyclophosphamide, Adriamycin, vincristine, methylhydrazine	3		5 (2 pts with 2 pregnancies)	on chemotherapy, $4\frac{1}{2}$, $5\frac{3}{4}$, $1\frac{4}{12}$, $6\frac{5}{12}$ yr
Blatt et al., 1981 [34]	Acute leukemia (ALL)	Prednisone, vincristine, methotrexate, 6-mercaptopurine	6	6 normozoospermia (21–150 mill sperm/ml)	1	3.5/4.5/5.0/5.8/ 6.0/8.7 yr
Brämswig et al., 1990 [3]	M. Hodgkin	OPPA/COPP	4	4 azoospermia	Not reported	5.8/6.2/7.2/8.3 yr
Dhabhar et al., 1993 [35]	M. Hodgkin	COPP/MOPP	26	18 azoospermia	Not reported	Median 72 months
Heyn et al., 1992 [36]	Paratesticular rhabdomyosarcoma	Chlorambucil	3	2 azoospermia 1 oligozoospermia	Not reported	9, 15, 13 yr
Meistrich et al., 1989 [37]	Osteosarcoma	Cisplatin, Adriamycin, dacarbazine	11	1 azoospermia 5 oligozoospermia 5 normozoospermia	Not reported	Range 7–37 yr
Shafford et al., 1993 [38]	M. Hodgkin	Chlorambucil, vincristine, procarbazine, prednisolone	13	10 azoospermia 1 oligozoospermia	Not reported	Median 11 yr 9 months, range 6–18 yr
Shamberger et al., 1981 [39]	Soft tissue sarcoma	Adriamycin, cyclophosphamide, methotrexate	4	3 azoospermia 1 oligozoospermia	None	8/27/29/32 months
1981 [40]	Osteosarcoma	Methotrexate, vincristine	2	2 normozoospermia	None	14/34 months
Siimes & Rautonen, 1990 [41]	Leukemia and solid tumors	Adriamycin, cisplatin (3–6 cycles preop., 6–12 cycles postop.)	46	20 azoospermia 17 oligozoospermia 9 normozoospermia	Not reported	Follow-up 14.5 yr
Wallace et al., 1991 [42]	Acute leukemia (ALL)	Vincristine, prednisolone, 6-mercaptopurine, methotrexate	19	5 azoospermia 14 normozoospermia	Not reported	Median 10.7 yr
Watson et al., 1985 [43]	Childhood nephrotic syndrome	Cyclophosphamide (2–3 mg/kg body weight for 42–556 days, mean 280 days)	30	4 azoospermia 9 oligozoospermia (6.3 ± 1.5 mill sperm/ml) 17 normozoospermia (54.5 ± 7.6 mill sperm/ml)	Not reported	Median 12.8 yr, range 6.7–5.8 yr
				3 azoospermia 6 oligozoospermia 21 normozoospermia	Not reported	After additional 7.2 yr of follow-up
Whitehead et al., 1982 [44]	M. Hodgkin	Mustine, vincristine, procarbazine, prednisolone	6	6 azoospermia	Not reported	Mean 5.3 yr, range 2.4–8 yr

*ALL = acute lymphoblastic leukemia; AML = acute myeloblastic leukemia; M. = morbus; pts = patients.

the first data to be presented on the semen characteristics of pubertal boys, whether healthy or diseased. It remains unclear whether the impairment of semen variables seen in half of the patients is mainly due to the pubertal status or to the underlying disease. The first question could be addressed by establishing an age-matched control group of pubertal healthy boys, which, unfortunately, is not yet available. The second question may be answered by a comparison of the results obtained in adolescent and adult males with malignancies. Subnormal semen parameters are fairly common in patients with testicular tumors and other malignancies, for example, Hodgkin's diseases, although the reasons for impairment of spermatogenesis by illness prior to any therapeutic intervention remains unclear [5,6,21,22].

The availability of techniques for assisted fertilization offers the possibility of inducing pregnancy in the female partner by using the cryopreserved semen [4,23,24]. However, the additional impairment of semen quality, especially sperm motility, by the process of cryopreservation represents further problems when cryopreserved semen is used in assisted fertilization. Thus improvement of cryopreservation techniques, for example, by swim-up preparation prior to cryopreservation, is important [25]. No data are available to evaluate the impairment of sperm due to long-term storage of 10–15 years after cryopreservation with modern techniques. However, the reported pregnancies induced by cryopreserved semen were achieved with both short- and long-term (e.g., 10 years) stored semen [4]. Thus we may speculate that storage itself under constant conditions may be not as important for sperm quality as the procedure of cryopreservation itself.

Thus far about 120 births have been documented worldwide after the use of semen cryopreserved from men with malignancies [4]. With improvement of in vitro fertilization techniques, and especially the possibility of performing intracytoplasmic sperm injection, the chances for achieving pregnancies by the use of cryopreserved semen from oncological patients will increase [4,26]. Even in patients with low sperm counts and low sperm motility, these techniques may be successful in inducing pregnancies in a female partner. However, to date no success rates are yet available. But this present lack of information should not prevent oncologists from recommending semen cryopreservation to patients, as these techniques used in assisted reproduction are being improved, and well-counselled patients may later profit from this development. Moreover, the pregnancies achieved either by sexual intercourse or assisted fertilization showed no increased risk of malformation in the newborn if either the father or mother had been treated for malignancies during childhood or adulthood [4,27–31]. Therefore, patients' fear of bearing malformed children, either by the use of semen cryopreserved during illness or by the use of semen obtained after aggressive therapy and recovered reproductive function, may be allayed by adequate counselling.

The use of cryopreservation of semen in adolescent boys and the application of assisted reproductive techniques for procreation in adulthood may contribute to improving the quality of life in long-term survivors of childhood malignancies. From our own experience we may conclude that 14- to 15-year-old boys, though differing in physical and psychological maturation, and their parents understand the possibility of preserving fertility that may be impaired by necessary oncological treatments and the possibility of contributing to the young boys' long-term quality of life. Masturbation itself seems not to be a major problem, as many boys experience their sexual development at an earlier age than their parents may be aware. Thus the preservation of fertility should be taken into consideration when young male patients are counselled within the scope of oncological therapy.

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